Baum *et al.*, 1990, and U. S. Patents 4,356,270; 4,362,817; 4,371,625, and 5,441,884, each incorporated specifically herein by reference.

The *B. thuringiensis* gene can be introduced between the transcriptional and translational initiation region and the transcriptional and translational termination region, so as to be under the regulatory control of the initiation region. This construct will be included in a plasmid, which will include at least one replication system, but may include more than one, where one replication system is employed for cloning during the development of the plasmid and the second replication system is necessary for functioning in the ultimate host. In addition, one or more markers may be present, which have been described previously. Where integration is desired, the plasmid will desirably include a sequence homologous with the host genome.

The transformants can be isolated in accordance with conventional ways, usually employing a selection technique, which allows for selection of the desired organism as against unmodified organisms or transferring organisms, when present. The transformants then can be tested for pesticidal activity. If desired, unwanted or ancillary DNA sequences may be selectively removed from the recombinant bacterium by employing site-specific recombination systems, such as those described in U. S. Patent 5,441,884 (specifically incorporated herein by reference).

2.4 SYNTHETIC *CRY1C** DNA SEGMENTS

A *B. thuringiensis cry1** gene encoding a crystal protein having insecticidal activity against Lepidopteran insects comprising a modified amino acid sequence in one or more loop regions of domain 1 or in a loop region between domain 1 and domain 2 represents an important aspect of the invention. Preferably, the *cry1** gene encodes an amino acid sequence in which one or more loop regions have been modified for the purpose of altering the insecticidal activity of the crystal protein. As described above, such loop domains include those between α helices 1 and 2, α helices 2 and 3, α helices 3 and 4, α helices 4 and 5, α helices 5 and 6, or α helices 6 and 7 of domain 1, or between α helix 7 of domain 1 and β strand 1 of domain 2 (FIG 1). Preferred *cry1** genes of the invention include *cry1A**, *cry1B**, *cry1C**, *cry1D**, *cry1E**, *cry1F**, *cry1G**, *cry1H**,

*cry1I**, *cry1J**, and *cry1K** genes, with *cry1Aa**, *cry1Ab**, *cry1Ac**, *cry1Ad**, *cry1Ae**, *cry1Ba**, *cry1Bb**, *cry1Bc**, *cry1Ca**, *cry1Cb**, *cry1Da**, *cry1Db**, *cry1Ea**, *cry1Eb**, *cry1Fa**, *cry1Fb**, *cry1Hb**, *cry1Ia**, *cry1Ib**, *cry1Ja**, and *cry1Jb** genes being highly preferred.

In accordance with the present invention, nucleic acid sequences include and are not limited to DNA, including and not limited to cDNA and genomic DNA, genes; RNA, including and not limited to mRNA and tRNA; antisense sequences, nucleosides, and suitable nucleic acid sequences such as those set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:58, and SEQ ID NO:60 and alterations in the nucleic acid sequences including alterations, deletions, mutations, and homologs capable of expressing the *B. thuringiensis* modified toxins of the present invention.

In an illustrative embodiment, the inventors used the methods described herein to produce modified *cry1Ca** genes which had improved insecticidal activity against lepidopterans. In these illustrative examples, loop regions were modified by changing one or more arginine residues to alanine or aspartic acid residues, such as mutations at arginine residues Arg148 and Arg180.

As such the present invention also concerns DNA segments, that are free from total genomic DNA and that encode the novel synthetically-modified crystal proteins disclosed herein. DNA segments encoding these peptide species may prove to encode proteins, polypeptides, subunits, functional domains, and the like of crystal protein-related or other non-related gene products. In addition these DNA segments may be synthesized entirely *in vitro* using methods that are well-known to those of skill in the art.

As used herein, the term "DNA segment" refers to a DNA molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a DNA segment encoding a crystal protein or peptide refers to a DNA segment that contains crystal protein coding sequences yet is isolated away from, or purified free from, total genomic DNA of the species from which the DNA segment is obtained, which in the instant case is the genome of the Gram-positive bacterial genus, *Bacillus*, and in particular, the species of *Bacillus* known as *B. thuringiensis*. Included within the term "DNA segment",

are DNA segments and smaller fragments of such segments, and also recombinant vectors, including, for example, plasmids, cosmids, phagemids, phage, viruses, and the like.

Similarly, a DNA segment comprising an isolated or purified crystal protein-encoding gene refers to a DNA segment which may include in addition to peptide encoding sequences, certain other elements such as, regulatory sequences, isolated substantially away from other naturally occurring genes or protein-encoding sequences. In this respect, the term "gene" is used for simplicity to refer to a functional protein-, polypeptide- or peptide-encoding unit. As will be understood by those in the art, this functional term includes both genomic sequences, operon sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides or peptides.

"Isolated substantially away from other coding sequences" means that the gene of interest, in this case, a gene encoding a bacterial crystal protein, forms the significant part of the coding region of the DNA segment, and that the DNA segment does not contain large portions of naturally-occurring coding DNA, such as large chromosomal fragments or other functional genes or operon coding regions. Of course, this refers to the DNA segment as originally isolated, and does not exclude genes, recombinant genes, synthetic linkers, or coding regions later added to the segment by the hand of man.

Particularly preferred DNA sequences are those encoding Cry1C-R148A, Cry1C-R148D, Cry1C-R180A, Cry1C.499, Cry1C.563 or Cry1C.579 crystal proteins, and in particular *cry1C** genes such as *cry1C-R148A*, *cry1C-R148D*, *cry1C-R180A*, *cry1C.499*, *cry1C.563* and *cry1C.579* nucleic acid sequences. In particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences that encode a Cry peptide species that includes within its amino acid sequence an amino acid sequence essentially as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:59, or SEQ ID NO:61.

The term "a sequence essentially as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:59, or SEQ ID NO:61" means that the sequence substantially corresponds to a portion of the sequence